

## Light Insensitive Silver(I) Cyanoximates As Antimicrobial Agents for Indwelling Medical Devices

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Ten silver(I) cyanoximates of AgL composition (L = NC-C(NO)-R, where R is electron withdrawing groups: -CN, -C(O)NR<sub>2</sub>, -C(O)R' (alkyl), -C(O)OEt, 2-heteroaryl fragments such as 2-pyridyl, 2-benzimidazolyl, 2-benzoxazolyl, 2-benzthiazolyl) were synthesized and characterized using spectroscopic methods and X-ray analysis. Crystal structures of four complexes were determined and revealed the formation of two-dimensional (2D) coordination polymers of different complexity in which anions exhibit bridging or combined chelate and bridging binding modes. In these compounds, anions are in the nitroso form. All studied AgL complexes are sparingly soluble in water and are thermally stable to 150 °C. Synthesized compounds demonstrated remarkable insensitivity toward visible light and UV-radiation, which was explained based on their polymeric structures with multiple covalent bonds between bridging cyanoxime ligands and Ag(I) centers. All 10 silver(I) cyanoximates were tested in vitro on the subject of their antimicrobial activity against both Gram-positive and Gram-negative microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* sp., *Pseudomonas aeruginosa*, *Enterococcus hirae*, *Streptococcus mutans*, *Staphylococcus aureus*, and *Mycobacterium fortuitum* as well as against *Candida albicans* in solutions, and in the solid state as pressed pellets and dried filter paper disks presoaked with solutions of AgL in DMF. Results showed pronounced antimicrobial activity for all investigated complexes. A combination of five factors: (1) light insensitivity, (2) poor water solubility, (3) high thermal stability, (4) lack of toxicity of organic ligands, and (5) in vitro antimicrobial activity allows development of silver(I) cyanoximates for medical applications. These include antimicrobial additives to acrylate glue, cured by UV-radiation, used in introduction of prosthetic joints and dental implants, and prevention of biofilm formation on several types of indwelling medical devices.

### Introduction

The number of patients requiring an internal fixation device or artificial joint has grown rapidly. In the United States alone, more than 4.4 million people have at least one internal fixation device and more than 1.3 million people have an artificial joint.<sup>1</sup> Bacterial infection induced by an implant placement (Scheme 1A) is a significant rising complication and is associated with considerable morbidity and costs.<sup>2</sup> These device-related infections caused by *Pseudomonas aeruginosa* are usually acute and extremely difficult to treat.<sup>3–5</sup> Initial symptoms of these infections

are usually transient,<sup>6</sup> allowing further infection development. Even immediate device replacement and long-term administration of high-dose antibiotics are often ineffective.<sup>7,8</sup> *Streptococcus mutans* is considered an early colonizer of the tooth surface and one of the major causes of caries development in humans. The ability of these bacteria to produce biofilms on the surfaces of biomaterials used during surgeries is one of the main causes of developing stubborn infections.<sup>9</sup> Biofilms<sup>10</sup> have an increased resistance to antibiotics<sup>11</sup> and host defenses.<sup>12,13</sup> As a result, they

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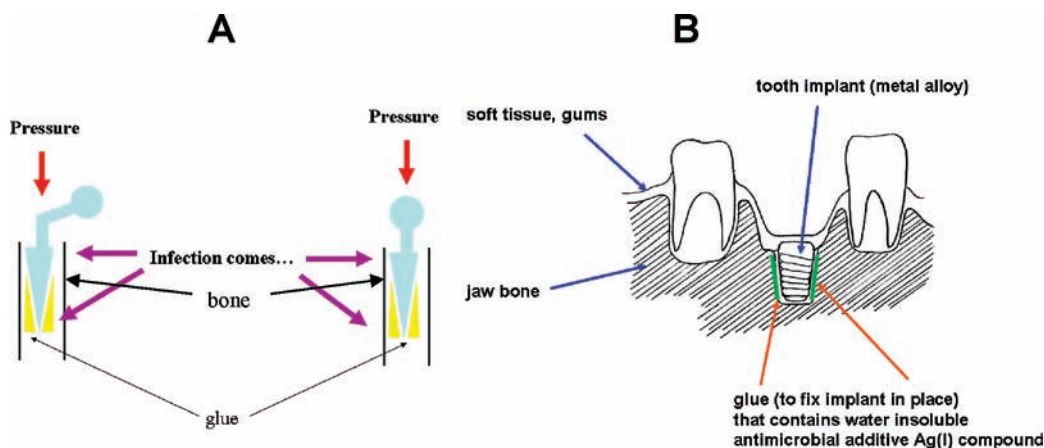
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Scheme 1



are notoriously difficult to eradicate, and are a source of many recurrent infections.

Clinical practice has shown that systemic antibiotics are unable to provide effective treatment for implant-associated infections. At the present time, only a high dose of antibiotics applied locally at the bone–implant interface can prevent such bacterial infections. However, this treatment causes a number of side effects, such as increased bacterial resistance to antibiotics, allergic reactions, and microbial flora depletion. Thus, it is often the best solution to remove the device, treat the infection, and repeat surgical introduction of a new device. This kind of procedure is costly, both financially and psychologically, as well as time-consuming.

Due to the rapid increase in use of artificial implants, it is critical that new infection-preventing strategies are developed, and in particular, antibacterial agents. One of the interesting options is the introduction of a non-antibiotic antimicrobial substance, for example, into the glue used during introduction of indwelling medical devices (Scheme 1) or surface treatment of such devices to prevent pathogenic cell adhesion and biofilm formation. There is a critical need for non-antibiotic compounds that satisfy specific needs to be water insoluble, light- and chemically stable, and yet could survive sterilization at 100 °C without decomposition. Pure organic compounds cannot meet these criteria, but some water-insoluble metal complexes – for example silver(I) compounds – can (Scheme 1B). Therefore, it is important to identify and study new metal-based compounds that will meet these demands. Silver has long been known to exhibit strong inhibitory and bactericidal effects as well as a broad spectrum of antibacterial properties. Since ancient times, people have known that water can remain suitable for drinking for a long time if stored in silver jars. Colloidal silver and silver nitrate have been used safely in burn therapy, urinary tract infections, and central venous catheter infections.<sup>14</sup> The inhibitory effects of silver and silver compounds on bacteria is believed to be associated with silver reacting with microbial DNA or the sulfhydryl groups found in the enzymes of bacterial electron transport chains, causing its inactivation.<sup>15</sup> One of the applications of silver and its compounds is reduction of postoperative infections caused by implants. Thus, intrinsically low toxicity silver compounds were loaded into several implant materials<sup>18</sup>

such as bioglass<sup>16</sup> and bone cement,<sup>17</sup> and therefore, have tremendous potential for implant therapies in the health care industry.<sup>19–21</sup> However, to the best of our knowledge, there are no silver(I) compounds that combine light and thermal stability, chemical inertness, water insolubility, and show antimicrobial activity. The main problems appear to be poor knowledge, insufficient development, and lack of subsequent studies of organic ligands to provide such properties. However, there is one particular class of low molecular weight organic compounds that can act as ligands for binding silver(I) cations and form complexes that will satisfy all the specific requirements outlined above. These compounds are oximes, which represent versatile organic molecules that were extensively used as excellent ligands in analytical,<sup>22</sup> inorganic,<sup>23–28</sup> and bioinorganic chemistry.<sup>29–31</sup> Furthermore, among oximes one specific group of substances called cyanoximes – compounds with the general formula  $\text{HO-N}=\text{C}(\text{CN})\text{-R}$  – represents a new, special class of biologically active molecules<sup>32</sup> that are also

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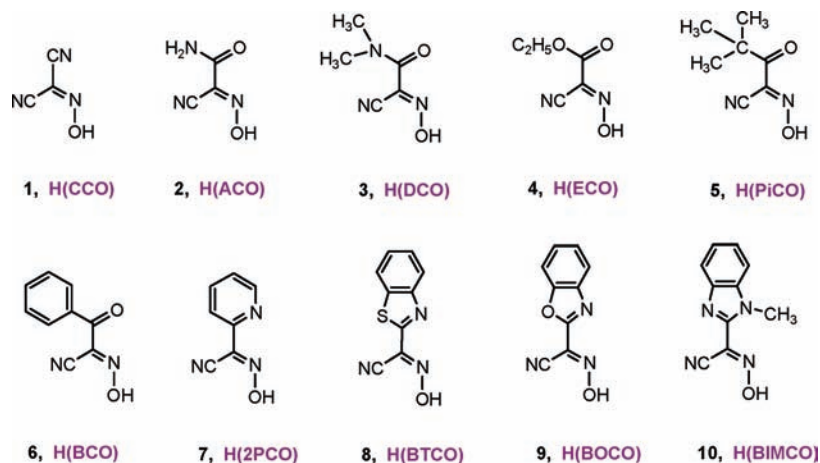
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Scheme 2



capable of binding to different metal ions.<sup>33–35</sup> The presence of the CN-group significantly increases their acidity and makes them better ligands for binding metal ions as compared to conventional monoximes. Earlier data showed no intrinsic in vitro cytotoxicity of free organic cyanoximes,<sup>36–38</sup> and we found that silver(I) cyanoximates are insoluble in water, represent thermally and chemically stable compounds,<sup>39–42</sup> and also exhibit antimicrobial activity.<sup>43</sup> With the exception of several publications and presentations,<sup>44–48</sup> there were no systematic studies regarding light-stable silver(I) antimicrobial

compounds targeting specific infections, and no oxime-based compounds were tested on that matter at all.

In this work, we present results of the first part of our systematic investigation dealing with the synthesis, spectroscopic, structural and photophysical characterization, as well as antimicrobial activity studies for a new group of light-insensitive silver(I) cyanoximates based on ligands shown in Scheme 2.

## Experimental Section

**Materials and Physical Measurements.** Reagent or analytical grade materials were obtained from commercial suppliers (Aldrich and Mallinckrodt) and were used without further purification. Elemental analyses on C, N, H content were performed by a combustion method at the Atlantic Microlab (Norcross, GA). Melting points for organic ligands were determined using the UniMelt apparatus (by Thomas-Hoover) without correction. Identification of the obtained organic compounds was carried out using <sup>1</sup>H, <sup>13</sup>C NMR spectroscopy (Varian INova 400; *T* = 296 K; in DMSO-*d*<sub>6</sub>, with TMS as an internal standard TMS, by Cambridge Laboratories), and mass-spectrometry (positive FAB technique for the macrocyclic compound 14-an[e]N<sub>4</sub>; *m*-nitrobenzyl alcohol, NBA, as a matrix using Autospec Q and ZAB spectrometers from Manchester, UK). IR spectra for synthesized organic cyanoxime ligands were recorded in KBr pellets (400–4000 cm<sup>-1</sup> region at 4 cm<sup>-1</sup> resolution) using a Nicolet Impact 410 spectrophotometer operating with OMNIC software. At the same time, IR spectra of silver(I) complexes were obtained from mulls in Nujol between two 2 cm KBr disks. Visible spectra for the suspensions of several Ag(I) cyanoximates in mineral oil between two quartz plates 4 × 1 cm were recorded on an Agilent HP 8453E spectrophotometer in the range of 300–1100 nm at 293 K. Room temperature solid-state diffusion reflectance spectra of Ag(ACO) and Ag(PiCO) were obtained on a Varian Bio-100 spectrophotometer with an integrating sphere and MgO as a standard. Electrical conductivity of 1 mM solutions of synthesized silver(I) cyanoximates in anhydrous DMSO was measured at 296 K using a YSI Conductance-Resistance meter model 34. Solutions of ammonium bromide, tetrabutylammonium bromide, and tetraphenyl-phosphonium bromide (as 1:1 electrolytes), and hydrazinium dichloride (as 1:2 electrolyte) were used for the electrode calibration.

**X-ray Crystallography.** Suitable crystals of compounds Ag(ACO), Ag(DCO), Ag(PiCO), and Ag(ECO) were mounted on a thin glass fiber on the goniometer head of a Bruker APEX 2 diffractometer equipped with a SMART CCD area detector. All data sets were collected at low temperature. The intensity data for suitable crystals of these compounds (Supporting Information, Figure S1) were collected in  $\omega$  scan mode using Mo tube

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Table 1. X-ray Analysis Experimental Data for Several Light Insensitive Ag(I) Cyanoximates

parameter	Ag(ACO)	Ag(DCO)	Ag(PiCO)	Ag(ECO)
formula	C <sub>3</sub> H <sub>2</sub> AgN <sub>3</sub> O <sub>2</sub>	C <sub>5</sub> H <sub>6</sub> AgN <sub>3</sub> O <sub>2</sub>	C <sub>7</sub> H <sub>9</sub> AgN <sub>2</sub> O <sub>2</sub>	C <sub>5</sub> H <sub>5</sub> AgN <sub>2</sub> O <sub>3</sub>
formula weight	219.95	248.00	261.03	248.98
crystal system	monoclinic	orthorhombic	monoclinic	triclinic
space group	<i>P</i> 2 <sub>1</sub> / <i>c</i> , No. 14	<i>F</i> dd2, No. 43	<i>P</i> 2 <sub>1</sub> / <i>c</i> , No. 14	<i>P</i> $\bar{1}$ , No. 2
<i>a</i> (Å)	11.735(3)	28.708(8)	13.967(2)	3.5875(9)
<i>b</i> (Å)	6.8313(15)	29.606(9)	5.9130(9)	7.2743(18)
<i>c</i> (Å)	6.3756(15)	3.6835(11)	10.2611(15)	13.617(3)
$\alpha$ (°)	90.00	90.00	90.00	82.497(3)°
$\beta$ (°)	95.654(3)	90.00	104.4740(10)°	84.531(3)°
$\gamma$ (°)	90.00	90.00	90.00	81.968(3)°
<i>V</i> (Å <sup>3</sup> )	508.6(2)	3130.7(16)	820.5(2)	347.78(15)
<i>Z</i>	4	16	4	2
<i>D</i> <sub>calc</sub> (g·cm <sup>-3</sup> )	2.872	2.105	2.113	2.378
<i>F</i> (000)	416	1920	512	240
$\mu$ (MoK $\alpha$ ) (mm <sup>-1</sup> )	3.87	2.52	2.41	2.85
Data Collection				
radiation	MoK $\alpha$	MoK $\alpha$	MoK $\alpha$	MoK $\alpha$
temperature, K	120(2)	120(2)	150(2)	120(2)
wavelength	0.71073	0.71073	0.71073	0.71073
2 $\theta$ <sub>max</sub> (°)	51.98	60.94	52.72	50.66
index ranges	-14 > <i>h</i> > 14 0 > <i>k</i> > 8 0 > <i>l</i> > 7	-40 > <i>h</i> > 40 42 > <i>k</i> > -42 -5 > <i>l</i> > 5	-17 > <i>h</i> > 17 -7 > <i>k</i> > 7 -12 > <i>l</i> > 12	-4 > <i>h</i> > 4 -8 > <i>k</i> > 8 -16 > <i>l</i> > 16
reflections collected	1472	11552	7803	3385
unique data	996	2376	1673	1275
discrimination	2 $\sigma$	2 $\sigma$	2 $\sigma$	2 $\sigma$
Structure Refinement				
data used	928	2376	1673	1275
parameters refined	83	102	145	121
<i>R</i> ( <i>F</i> ), <i>wR</i> <sub>2</sub> ( <i>F</i> <sup>2</sup> ) (obs.)	0.024, 0.0275	0.0193, 0.0392	0.0146, 0.0364	0.0279, 0.0677
<i>R</i> ( <i>F</i> ), <i>wR</i> <sub>2</sub> ( <i>F</i> <sup>2</sup> ) (all data)	0.0609, 0.0699	0.0232, 0.0404	0.0170, 0.0377	0.0319, 0.0702
GOF on <i>F</i> <sup>2</sup>	1.209	1.029	1.116	1.033
largest peak/hole (e Å <sup>-3</sup> )	1.05/-0.74	0.443/-0.393	0.527/-0.294	1.034/-0.927

(K $\alpha$  radiation;  $\lambda = 0.71073$  Å) with a highly oriented graphite monochromator. Intensities were integrated from four series of 364 exposures, each covering 0.5° in  $\omega$  within 20 to 60 s of acquisition time and the total data set being a sphere.<sup>49</sup> The space group determination was done with the aid of XPREP software.<sup>50</sup> Absorption corrections were applied based on crystal face indexing obtained using actual images recorded by video camera. The following data processing was performed using the SADABS program that was included in the Bruker AXS software package.<sup>51</sup> The structures were solved by direct methods and refined by least-squares on weighted *F*<sup>2</sup> values for all reflections using the SHELXTL program.<sup>50</sup> All atoms received assigned anisotropic displacement parameters and were refined without positional constraints. All nine hydrogen atoms in the structure of Ag(PiCO) were found on the difference map and their positions were refined as well. Crystals of Ag(ACO) turned out to be multidomain species and details of its structure solution are shown in Supporting Information, Figure S2. Complex Ag(DCO) contained a disordered solvent molecule with partial occupancy. Details of structure solution for this compound can be found in Supporting Information, Figure S2 as well. Crystal data for all four studied compounds are presented in Table 1, while bond lengths and valence angles are summarized in Table 2. A complete set of bonds and angles around metal centers is presented in Supporting Information, Figures S3–S7. Figures for the crystal structures of these complexes were drawn using Mercury 4.1.2 and ORTEP 32 software<sup>52</sup> at a

50% thermal ellipsoids probability level. The PLATON checks of crystallographic data and actual CIF files for reported structures can be found in the Supporting Information section. The X-ray powder diffraction studies of bulk samples of synthesized AgL were carried out at 296 K on a Bruker Discover XRD system using Cu-radiation (K $\alpha$  radiation;  $\lambda = 1.5406$  Å). Powdery samples of complexes were attached to 20 × 45 mm cardboard rectangles (as sample holders) using a double-sided 12 mm Scotch tape from 3M followed by standard one-sided 19 mm tape.

**Synthesis of Compounds.** Preparation of cyanoximes<sup>37,53</sup> and their Ag(I) complexes is depicted in Scheme 3, while the typical synthesis of silver(I) derivatives is shown as an example for only one compound.

**2-(Oximido)-2-benzoxazoleacetonitrile Silver(I), Ag(BOCO).** Brown H(BOCO) in the amount of 0.4605 g (2.46 mM) of brown H(BOCO) was dissolved in a mixture of 10 mL of EtOH, diluted with 10 mL of water, heated to +50 °C, and then added dropwise to a solution of 0.169 g (1.22 mM) of K<sub>2</sub>CO<sub>3</sub> in 10 mL of H<sub>2</sub>O. The reaction mixture turned immediately very dark brown and was placed for 2 min into an ultrasound bath to accelerate the evolution of CO<sub>2</sub>. A solution of 0.4183 g (2.46 mM) of AgNO<sub>3</sub> in 10 mL of water was added dropwise under intensive stirring to a solution of K(BOCO) above. Mixing resulted in a very fine orange-brown precipitate, which after an additional 20 min of stirring was filtered, washed with three portions of 10 mL of water, and then dried in a vacuum desiccator charged with H<sub>2</sub>SO<sub>4</sub> (c) for 3 days. The yield of orange-brown Ag(BOCO) was 98% (0.701 g). Anal. Calc. for C<sub>9</sub>H<sub>4</sub>AgN<sub>3</sub>O<sub>2</sub> (Found) %: C, 36.77 (37.12); H, 1.37 (1.58); N, 14.49 (14.33). Other silver(I) cyanoximates follow.

**Silver(I) Nitrosodicyanomethanide, Ag(CCO).** Bright-yellow powder; yield 96%, complex decomposes in the range of

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190–209 °C. Anal. Calc. for C<sub>3</sub>N<sub>3</sub>OAg (Found) %: C, 17.84 (17.98); N, 20.81 (20.69).

**Silver(I) α-Oximido-(acetamide)acetonitrile, Ag(ACO).** Microcrystalline canary-yellow compound; yield 100%, decomposition > 229–232 °C. Anal. Calc for C<sub>3</sub>H<sub>2</sub>AgN<sub>3</sub>O<sub>2</sub> (Found): C, 16.38 (16.47); H, 0.92 (0.99); N, 19.11(19.08).

**Silver(I) α-Oximido-([N,N-dimethylamine]acetamide)acetonitrile, Ag(DCO)·0.5MeOH.** Dark-yellow microcrystalline compound obtained with 82% yield, decomposes at ~158 °C. Anal. Calc for C<sub>11</sub>H<sub>16</sub>Ag<sub>2</sub>N<sub>6</sub>O<sub>5</sub> (Found): C, 25.02 (25.11); H, 3.05 (3.09); N, 15.92 (15.63).

**Silver(I) α-Oximido-(ethylacetoxy)acetonitrile, Ag(ECO).** Orange powder, 93% yield, decomposes at 202–207 °C. Anal. Calc for C<sub>5</sub>H<sub>5</sub>AgN<sub>2</sub>O<sub>3</sub> (Found): C, 24.12 (23.98); H, 2.02 (2.06); N, 11.25 (11.11).

**Silver(I) α-Oximido-(2-pivaloyl)acetonitrile, Ag(PiCO).** Very fine pale yellow powder, yield 85%; rapid decomposition at

160–162 °C. Anal. Calc for C<sub>7</sub>H<sub>9</sub>AgN<sub>2</sub>O<sub>2</sub> (Found): C, 32.21 (31.81); H, 3.48 (3.51); N, 10.73 (10.62).

**Silver(I) α-Oximido-(2-benzoyl)acetonitrile, Ag(BCO).** Purple fine powder obtained with yield 71%; decomposition at 180–182 °C. Anal. Calc for C<sub>9</sub>H<sub>5</sub>AgN<sub>2</sub>O<sub>2</sub> (Found): C, 38.47 (38.29); H, 1.79 (1.92); N, 9.97 (9.86).

**Silver(I) α-Oximido-(2-pyridyl)acetonitrile, Ag(2PCO).** Yellow powder, yield 87%; decomposition at 178–187 °C. Anal. Calc for C<sub>7</sub>H<sub>5</sub>N<sub>3</sub>O (Found): C, 33.10 (34.83); H, 1.59 (1.90); N, 16.54 (17.18).

**Silver(I) α-Oximido-(2-[N-methyl]benzimidazolyl)acetonitrile, Ag(BIMCO)·0.5H<sub>2</sub>O.** The compound represents a very fine yellow-green powder, decomposes at 160–180 °C; yield 96%. Anal. Calc for C<sub>10</sub>H<sub>8</sub>AgN<sub>4</sub>O<sub>1.5</sub> (Found): C, 37.97 (37.60); H, 3.16 (2.47); N, 17.72 (17.20).

**Silver(I) α-Oximido-(2-benzothiazolyl)acetonitrile, Ag(BTCO)·1.5H<sub>2</sub>O.** Orange-brown fine powder; yield 100%; at ~214–219 °C complex decomposes. Anal. Calc for C<sub>9</sub>H<sub>7</sub>N<sub>3</sub>O<sub>2</sub>SAg, %: C, 32.03 (32.18); H, 2.08 (2.31), N, 12.46 (12.86).

All synthesized Ag(I) cyanoximates are sparingly soluble in water, but dissolve in donor solvents such as pyridine, 2-picoline, and DMSO.

**Photophysical Measurements.** Light sensitivity and stability of synthesized Ag(I) cyanoximates was studied at 296 K using a low-pressure Hg lamp with > 85% output at λ<sub>max</sub> = 254 nm where the intensity of light was measured with a UVX radiometer (UK). After 4 min warm-up time, this lamp generated a steady flux of UV-radiation that was equal to the dose of 10.13 J/cm<sup>2</sup> within 30 min. Prior to these investigations, complexes were thoroughly dried under high vacuum (<10<sup>-4</sup> Torr) and ground into a powder using an agate mortar. This made it easy to apply these compounds to paper, adhesive tape, or cardboard. Samples of complex were spread on white cardboard with an exposed circular area (via a mask) of ~3 cm. The radiation source was positioned 1 cm from the sample. A pristine white silver chloride prepared in the dark was used as a control substance to access the light stability of silver(I) cyanoximates. Digital pictures of irradiated samples were taken every 30 min using a stationary digital camera (Kodak DX7630; 6.1 Mpx), and native images were cropped to square shape without any contrast or brightness editing using Samsung Digimax Viewer 2.0 software. The experimental setup is shown in Figure S8, Supporting Information.

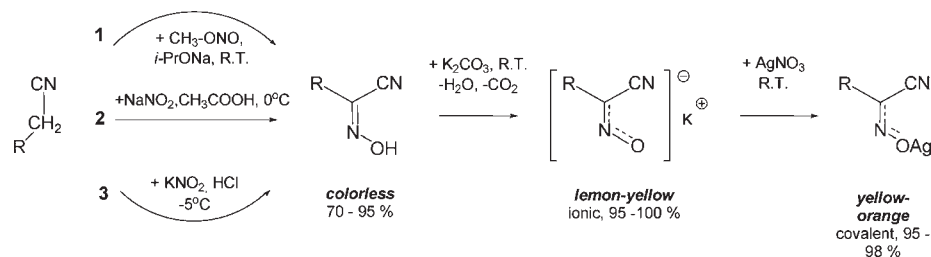
**Antimicrobial Activity Studies. Bacterial Strains and Growth Conditions.** The strains of bacteria were obtained from Polish Collection of Microorganisms (PCM) of the Institute of Immunology and Experimental Therapy of Polish Academy of Sciences and were used throughout this study. Both Gram-negative and Gram-positive bacteria, as well as yeasts, commonly isolated from patients suffering implant-related infections, were chosen for experiments. They included *Escherichia coli* PCM 1144 (ATCC #10536), *Klebsiella pneumoniae* PCM 57, *Proteus* sp. PCM 542 (ATCC# 13315), *Pseudomonas aeruginosa* PCM 2563 (ATCC #15442), *Streptococcus mutans* PCM 2502, *Staphylococcus aureus* PCM 2602 (ATCC #6538), *Enterococcus hirae* PCM 2559 (ATCC #10541), *Mycobacterium fortuitum* PCM 672, *Candida albicans* PCM 2566 (ATCC #10231).

**Table 2.** Selected Bond Lengths and Valence Angles for Cyanoxime Anions in Silver(I) Complexes

complex	bonds, Å	angles, °
Ag(ACO)	C(1)–N(1) = 1.311(7)	N(1)–C(1)–C(2) = 117.1(4)
	C(1)–C(2) = 1.437(7)	N(1)–C(1)–C(3) = 120.2(4)
	C(1)–C(3) = 1.484(7)	C(2)–C(1)–C(3) = 122.7(4)
	C(2)–N(2) = 1.323(7)	N(2)–C(2)–C(1) = 118.7(5)
	C(3)–O(2) = 1.323(7)	O(2)–C(2)–N(2) = 122.0(5)
	C(3)–N(3) = 1.151(7)	O(2)–C(2)–C(1) = 119.3(4)
	N(1)–O(1) = 1.307(5)	N(3)–C(3)–C(1) = 179.7(6)
Ag(DCO)	O(1)–N(1)–C(1) = 118.3(4)	
	N(1)–C(1)–C(2) = 119.82(19)	
	N(1)–C(1)–C(3) = 116.66(18)	
	C(2)–C(1)–C(3) = 122.93(18)	
	N(2)–C(2)–C(1) = 177.1(3)	
	O(1)–C(3)–N(3) = 121.67(18)	
	O(1)–C(3)–C(1) = 119.09(18)	
Ag(PiCO)	N(3)–C(3)–C(1) = 119.23(17)	
	O(2)–N(1)–C(1) = 118.45(19)	
	C(5)–N(3)–C(4) = 116.69(16)	
	N(1)–C(1)–C(2) = 116.80(16)	
	N(1)–C(1)–C(3) = 117.52(15)	
	C(2)–C(1)–C(3) = 125.67(15)	
	N(2)–C(2)–C(1) = 170.85(19)	
Ag(ECO) <sup>a</sup>	O(2)–C(3)–C(1) = 119.17(15)	
	O(2)–C(3)–C(4) = 121.45(16)	
	C(1)–C(3)–C(4) = 119.20(15)	
	O(1)–N(1)–C(1) = 118.10(15)	
	N(1)–C(1)–C(2) = 119.4(4)	
	N(1)–C(1)–C(3) = 120.5(4)	
	C(2)–C(1)–C(3) = 120.1(4)	
Ag(ECO) <sup>a</sup>	N(2)–C(2)–C(1) = 179.4(5)	
	O(2)–C(3)–O(3) = 124.0(4)	
	O(2)–C(3)–C(1) = 118.5(4)	
	O(3)–C(3)–C(1) = 117.6(4)	
	O(2)–C(4)–C(5) = 106.3(6)	
	O(1)–N(1)–C(1) = 116.8(3)	
	C(3)–O(2)–C(4) = 114.2(4)	

<sup>a</sup>Data for disordered mirrored ethoxy group are not present; for complete details on disorder see CIF file in Supporting Information.

### Scheme 3



In addition, several multidrug-resistant bacterial strains isolated from patients with nosocomial infections *K. pneumoniae* 244, *P. aeruginosa* 2314, AMA, *Enterococcus faecium* VRE (vancomycin-resistant), *Streptococcus pneumoniae* PCI, *S. aureus* MRSA (methicillin-resistant), *S. aureus* MRSC (coagulase-negative methicillin-resistant), were also included in the in vitro testing. Bacteria were cultivated on sugar broth at 37 °C for 24 h in aerobic conditions, and prior to use in tests, cells were diluted with the same medium to obtain suspensions of about  $2 \times 10^5$  cfu/mL.

**Antibacterial Tests in Solutions.** The antibacterial activities of synthesized Ag(I) cyanoximates were determined against bacterial strains by the microplate Alamar Blue assay.<sup>54</sup> Stock solutions of AgL compounds were prepared in DMSO (1 mg/mL) and then were diluted with appropriate media in the range from 0.03 to 1000  $\mu\text{g/mL}$  on the cell culture microtitration plate. Aliquots of 100  $\mu\text{L}$  of the studied AgL compound at different concentrations and 100  $\mu\text{L}$  of the diluted suspension of the bacterial cells were added to wells. Control wells contained either bacteria only or medium only, and plates were incubated at 37 °C for 48 h. After that, solutions of 20  $\mu\text{L}$  of Alamar Blue reagent (AbD Serotec) (10 $\times$  diluted) and 12.5  $\mu\text{L}$  of 20% Tween 80 were added to wells and incubation was continued at 37 °C for 2 more hours. The fluorescence was measured using Victor<sup>2</sup> apparatus (Wallac, Perkin-Elmer), and the experiment was repeated three times. The minimal inhibitory concentration (MIC) was defined as the lowest drug concentration which prevented a color change from blue to pink, inhibiting the bacterial growth for  $\geq 90\%$ . Means and standard error values were determined using the Microsoft Excel.

**Antibacterial Tests in Solid State: Soaked Dried Paper Disks and Pellets.** Solutions (or suspensions if the compound was not completely solubilized) of studied Ag(I) cyanoximates in DMF at 10 mg/mL concentration were prepared and 20  $\mu\text{L}$  of it was placed on filter paper discs (diameter 12 mm). These discs were dried for  $\sim 2$  h in the dark prior to testing where each filter was placed on agar plates with inoculated microorganisms, which were then incubated at 37 °C for 24 h. Thereafter, microorganisms' growth inhibition zone (if present) was measured (in mm).

Also, Ag(I) cyanoximates were pressed into 12 mm in diameter pellets using a Carver 20 tons hydraulic press. The method of testing pellets was similar to the one described above. Solid pellets of AgL were carefully placed on agar plates with inoculated bacteria. The exposition time was 24 h at 37 °C, after which inhibition zones were measured and results were documented by digital photography.

## Results and Discussion

Ten light insensitive silver(I) cyanoxime complexes with ligands shown in Scheme 2 were synthesized and characterized using spectroscopic methods, X-ray analysis, and in vitro antimicrobial studies. Below we present a description of properties of the obtained compounds in a systematic way starting from the chemical part of this work, continued by photo-physical studies, and then followed by biological investigations.

**Chemical Part: Synthesis and Characterization of Ligands and Metal Complexes.** Recently, we successfully developed a methodology for a high-yield preparation of cyanoximes from substituted acetonitriles.<sup>55,56</sup> These

R-CH<sub>2</sub>-CN compounds are mostly commercially available pure substances which can be converted in a one-step procedure to respective cyanoximes (Scheme 3). These are three different routes of the Meyer reaction,<sup>57</sup> which depending on the reactivity of the methylene group of starting acetonitrile were applicable for the preparation of different cyanoximes. Room temperature nitrosation **1** using gaseous methylnitrite CH<sub>3</sub>-ONO is the most convenient reaction that has been successfully used for synthesis of a variety of cyanoximes.<sup>56,58,59</sup> These compounds represent nice crystalline substances<sup>60</sup> that can be easily purified by recrystallization in the presence of activated carbon, or by flash column chromatography on silica.

Identification and characterization of the obtained and purified organic molecules were carried out using elemental analyses, thin-layer chromatography (TLC), and spectroscopic methods such as UV-visible, IR, <sup>1</sup>H, <sup>13</sup>C NMR spectroscopy (including 2D COSY, HMQC, and HSQC experiments). Ligands shown in Scheme 2 have the ability to form chelate complexes and also act as bridging ligands. They possess interesting features – electronic and structural – that are reflected in their hydrophilic/hydrophobic character and acidity (ligands **1–6**, Scheme 2), the ability to form layered structures in the solid state due to  $\pi$ - $\pi$  stacking interactions between heterocyclic groups (ligands **7–10**, Scheme 2), and, finally, multiple donor centers capable of tightly binding silver(I) atoms to form coordination polymers of different complexity. Coordination compounds of the latter metal are particularly interesting: they have unusual structures,<sup>61</sup> show properties of gas-sensing materials,<sup>41,42</sup> and lately demonstrate antimicrobial activity.<sup>43</sup> These aforementioned electronic and structural factors allow the fine-tuning of properties of the obtained Ag(I) complexes and possibility for study of structure–activity relationships during investigations of their in vitro biological activity.

Silver(I) cyanoximates with ligands shown in Scheme 2 have 1:1 stoichiometry and are poorly soluble in aqueous/alcohol solutions and thus can be conveniently separated from the reaction mixture. These AgL complexes are also insoluble in hydrocarbons, acetone, but are sparingly soluble in CH<sub>3</sub>CN. Dry, solid Ag(I) cyanoximates have shown significant thermal stability and do not decompose upon heating to 150 °C.

Visible spectra of fine suspensions of studied Ag(I) cyanoximates in mineral oil revealed broad ligand-based single band in the range of 400–500 nm that corresponds to  $n \rightarrow \pi^*$  transition in the nitroso chromophore. Obtained data of electron spectroscopy allowed estimation of the band gap in AgL, which turned out to be in the range of 3.00–3.55 eV (Figure S10, Supporting

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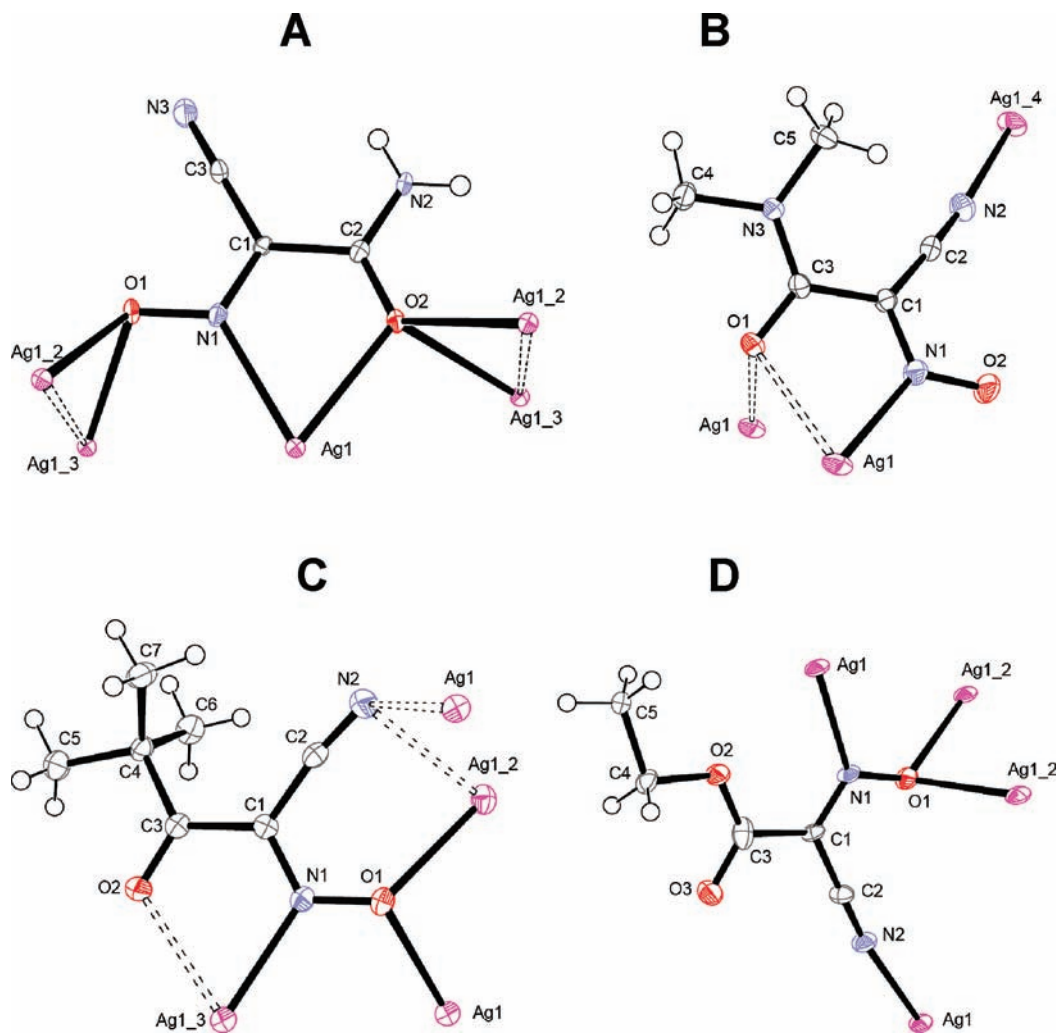
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**Figure 1.** Numbering schemes in crystal structures of silver(I) cyanoximates. Displayed are one anion and its closest Ag(I) environments in Ag(ACO) – A, Ag(DCO) – B, Ag(PiCO) – C, Ag(ECO) – D. An ORTEP drawing at 50% thermal ellipsoids probability level. Disordered methanol molecules in the structure of Ag(DCO) and mirror image of the disordered ethoxy-group in the structure of Ag(ECO) are not shown for clarity.

Information). What is the most important for current studies is that all synthesized AgL are light insensitive as was shown after many years of their exposure to daylight without change (Figure 4), and also to the short wavelength (254 nm) UV radiation (Figure 5). At that point it was clear that the explanation for a remarkable light insensitivity of these complexes rests in their solid-state structures. Despite the poor solubility of compounds in water, alcohols, acetone, and other common organic solvents, we were able to grow single crystals of Ag(I) cyanoximates suitable for the X-ray analysis. A thermostat with slow cooling of hot saturated AgL solutions within 5–6 days was used for crystal growth.

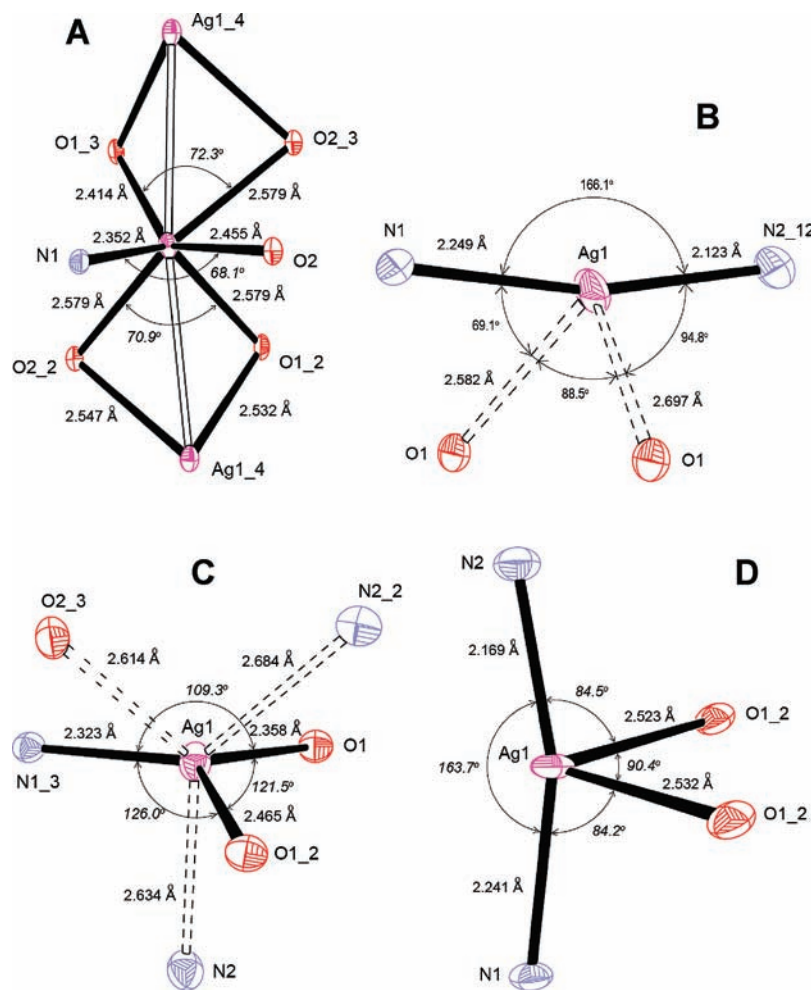
**Crystal Structures.** Details of determined crystal structures of Ag(ACO), Ag(DCO), Ag(PiCO), and Ag(ECO) are shown in Figures 1, 2, and 3 below in a way rather traditional for inorganic chemistry: the one cyanoxime ligand and closest metal centers, coordination polyhedron of silver(I) cation, and organization of the crystal lattice, respectively. All these compounds represent coordination polymers of different complexity in which the metal center is covalently bound to ligands since Ag–O and Ag–N distances are significantly shorter than the

sum of van der Waals radii of involved atoms equal to 3.24 and 3.27 Å,<sup>62</sup> and even ionic radii of 2.58 and 2.97 Å, respectively.<sup>62</sup> The only basic description of crystal structures is given below, while the complete set of bond lengths and valence angles – especially around metal centers – can be found in CIF files and Supporting Information (Figures S3–S7).

**Ag(ACO).** This complex represents layered 2D coordination polymer in which cyanoxime anion acts as both a chelating ligand and a bridging group utilizing oxygen atoms of the amide and nitroso groups (Figure 1A). Bond lengths in a planar five-membered chelate ring are Ag(1)–N(1) = 2.352 and Ag(1)–O(2) = 2.455 Å with the “bite angle” N(1)–Ag(1)–O(1) = 68.11°. The anion in the structure of Ag(ACO) is planar, in the nitroso form and adopts *cis-anti* configuration. This configuration of the *oxime* anion is contrary to the *trans-anti* found in organotin(IV),<sup>37</sup> organotellurium(IV),<sup>63</sup> and organoantimony(V)<sup>55</sup> complexes, but similar to that observed in

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**Figure 2.** Geometries of coordination polyhedrons in silver(I) cyanoximates: A – Ag(ACO), B – Ag(DCO), C – Ag(PiCO), D – Ag(ECO).

Ni(II) complexes,<sup>64</sup> where the ligand is in the nitroso form as well. The peculiarity of this structure is rather short “argentophilic” interactions at 3.1934(8) Å between Ag(I) centers in the 2D polymer propagating along the *c* axis (Figure 3). For comparison, the Ag–Ag single bond is 2.889 Å,<sup>65</sup> while the Ag---Ag distance in metallic silver (Fm3m; f.c.c.) is 4.086 Å.<sup>66</sup> With consideration of argentophilic interaction the coordination number of silver(I) center in Ag(ACO) is eight (Figure 2A). The H-bonding in the amide group is important for the stabilization of the structure since the only one hydrogen atom of the amide group in the anion is engaged in interlayer H-bonding, while the second hydrogen is involved in intralayer H-bonding with the oxygen atom of the nitroso group of neighboring anion.

**Ag(DCO).** This complex also represents a 2D layered coordination polymer (Figure 3B) but differently organized than that in the structure of Ag(ACO). Thus, the anion acts as a bridge between two silver(I) centers rather than a chelator (Figure 1B). Contrary to the structure of Ag(ACO), the CN-group of the anion in the structure of Ag(DCO) is involved in coordination to the metal center.

The anion is not planar, and there are two planes in its structure in the complex: O(2)–N(1)–C(1)–C(2)–N(2) and C(5)–N(3)–C(4)–C(3) with a dihedral angle of 40.05° between them. The oxygen atom O(1) is off the latter plane by 0.245 Å. The DCO<sup>−</sup> anion forms a conditional chelate ring with Ag atom: there is no planarity in the Ag(1)–N(1)–C(1)–C(3)–O(1) ring, and the distance Ag(1)–O(1) (2.582 Å, Figure 1) represents a long interaction between these atoms. Moreover, the oxygen atom O(1) acts as a bridge between two silver(I) atoms, and its other contact to the metal center is in 2.697 Å in length. Also, O(1) atom is off the plane Ag(1)–N(1)–O(2)–C(1)–C(3) by 0.596 Å, which is another indication of its electrostatic involvement into coordination to the silver atom. Thus, the coordination number of Ag(I) in the complex is two, with two long electrostatic contacts from the oxygen atom of the amide group (Figure 2B). The anion is in the nitroso form and adopts *cis-anti* configuration similar to that in its Cu(II) complex,<sup>67</sup> which is opposite to the *trans-anti* configuration found in structures of HDiCO<sup>68</sup> and Ph<sub>4</sub>Sb(DCO).<sup>55</sup> The bond length Ag(1)–N(2) is a record short – 2.128 Å, and followed by bond Ag(1)–N(1) = 2.217 Å (Figure 2B). The almost

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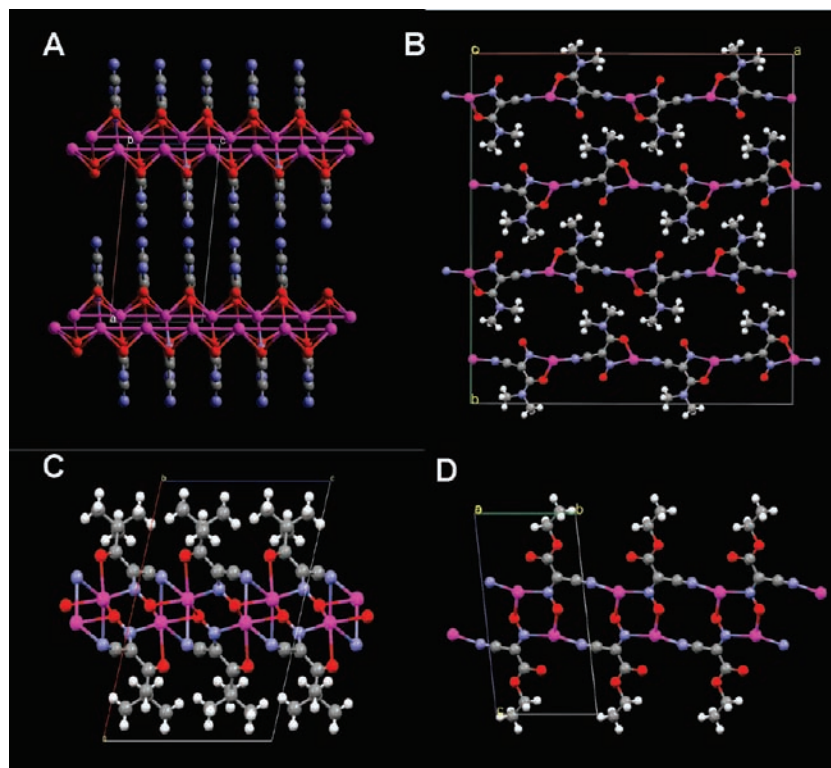
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**Figure 3.** Organization of covalent 2D-polymers in the structures of Ag(ACO) (A), Ag(DCO) (B), Ag(PiCO) (C), and Ag(ECO) (D). H-atoms of the amide group in the structure of Ag(ACO) are omitted for clarity. Coloring scheme: magenta – Ag, gray – C, red – O, blue – N.

linear arrangement between them at the angle  $\text{N}(1)\text{--Ag}(1)\text{--N}(2) = 166.11^\circ$  is responsible for the formation of polymeric chains which run along the  $c$  direction and joined together by bridging O(1) atom (with long contacts  $\text{Ag}(1)\text{--O}(1)$  and the angle  $\text{O}(1)\text{--Ag--O}(1)' = 88.48^\circ$  between chains) into an elegant 2D layered structure (Figure 3B). In the crystal, there are also van der Waals interactions between methyl groups of adjacent layers. Silver(I) atoms form linear arrays parallel to the  $c$  direction with intermetallic separation of 3.684 Å – longer than any reasonable metallophilic interactions.

**Ag(PiCO).** The crystal structure of this compound is a layered 2D coordination polymer as well (Figure 3C). The anion performs bridging function between four different silver(I) atoms (Figure 1C). Similar to the structure of Ag(ACO), the oxygen atom of the nitroso-group acts as a bridge between two metal centers (Figure 1C). There are three short covalent bonds in the coordination polyhedron: two bonds between Ag(I) and oxygen atoms O(1) and O(1)' of this bridging nitroso-group and the nitrogen atom of this group (Figures 1C and 2C). The distance  $\text{Ag}(1)\text{--O}(2) = 2.614$  Å is even longer than that in the structure of Ag(DCO) discussed above. Again, there is long interaction between these two atoms in  $\text{Ag}(1)\text{--N}(1)\text{--C}(1)\text{--C}(3)\text{--O}(2)$  arrangement, which is non-planar with the dihedral angle between mean planes  $\text{Ag}(1)\text{--O}(2)\text{--C}(3)$  and  $\text{Ag}(1)\text{--N}(1)\text{--C}(1)$  equal to  $21.25^\circ$ . This situation is similar to the structure of Ag(DCO), and it should be mentioned that in chelate complexes, five-membered rings are typically planar. The anion is in the nitroso-form (bond length  $\text{N}(1)\text{--O}(1)$  is shorter than  $\text{C}(1)\text{--N}(1)$ , Table 2) and adopts *cis-anti* configuration. The cyano group is bent (angle  $\text{C}(1)\text{--C}(2)\text{--N}(2) = 170.85^\circ$ ) and

reflects its unusual bridging role between two metal centers: the angle  $\text{Ag}(1)\text{--N}(2)\text{--Ag}(1)' = 84.95^\circ$  (Figure 1C). The nitrogen atom N(2) is not coordinated to these silver atoms due to geometrical restrictions imposed by its lone pair situated further along the  $\text{C}(2)\text{--N}(2)$  bond. Thus, angles  $\text{Ag}(1)\text{--N}(2)\text{--C}(2)$ ,  $\text{Ag}(1)'\text{--N}(2)\text{--C}(2)$ , and  $\text{Ag}(1)\text{--N}(2)\text{--Ag}(1)'$  are  $90.65^\circ$ ,  $118.38^\circ$ , and  $84.95^\circ$  respectively, and are highly improper in value to be considered for binding of the cyano group. For comparison, the angle  $\text{Ag--N}(5)\text{--C}(5)$  in the linearly coordinated CN-group in the structure of Ag(DCO) is  $164.65^\circ$ , while a similar angle  $\text{Ag}(1)\text{--N}(2)\text{--C}(2)$  is  $171.26^\circ$  in the structure of Ag(ECO) (Figure 2D). Therefore, coordination polyhedron of the Ag(I) center in Ag(PiCO) has a slightly distorted and practically planar trigonal geometry (Figure 2C). 2D layers in the structure of the complex are run along the  $c$  direction. Van der Waals interactions between *t*-butyl groups of anions exist between layers (Figure 3C). The shortest Ag---Ag distance in this complex is 3.591 Å.

**Ag(ECO).** This is the first known structure containing this cyanoxime ligand. The crystal structure of this complex follows already described Ag(DCO) and Ag(PiCO) and also represents layered 2D coordination polymer (Figure 3D). The ethoxy group in this complex is disordered by two positions with almost equal occupancies: 0.497 and 0.503. **Ag(ECO) structures presented** in Figures 1–3 structures contain only a major domain with its mirror image along  $\text{C}(1)\text{--C}(3)$  direction omitted for clarity. The  $\text{ECO}^-$  anion in this complex also acts as a bridging ligand between four different silver(I) centers (Figure 1D). All four bonds between the Ag(I) center and surrounding anions are covalent with those to nitrogen atoms N(1) and N(2) being especially short (Figure 2D). Coordination polyhedron of



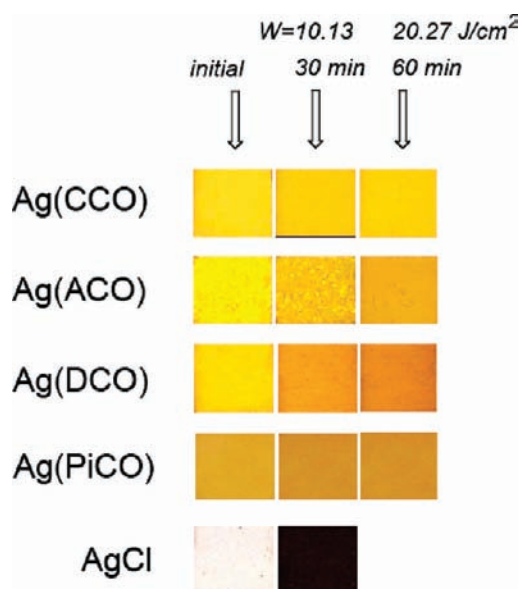
**Figure 4.** Vials with silver(I) cyanoximates showing the time of exposure to daylight.

the metal center in this complex is well described as a seesaw. The anion is in the nitroso-form (Table 2) and adopts a surprisingly planar *trans-anti* configuration of its cyanoxime core. The shortest Ag---Ag distance here is 3.587 Å.

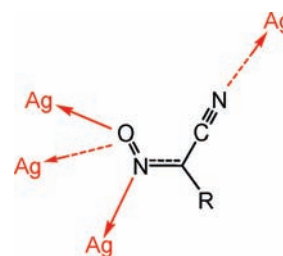
Determined single crystal structures of **silver(I) cyanoximates** described here silver(I) cyanoximates truly reflect structures of bulk materials since their calculated and experimental powder XRD patterns are in a good agreement (Figures S22 and S23, Supporting Information).

**Photophysical Part: UV-Light Stability Studies.** Silver(I) cyanoximates with chelating ligands shown in Scheme 2 exhibited a remarkable visible light insensitivity for years of direct exposure to daylight (Figure 4). This convenient property coupled with antimicrobial activity makes synthesized complexes potentially valuable materials for their investigations as additives to acrylate glues during introduction of indwelling medical devices such as prosthetic joints and dental implants (Figure S9, Supporting Information). These polymeric composites are cured within several minutes with UV-light that causes cross-linking and solidifying of the mass into a hard material. Therefore, studies of stability of Ag(I) cyanoximates toward short wavelength UV-radiation validated their choice for further practical considerations. Results of testing are shown in Figure 5 and indicate great stability of examined silver(I) cyanoximates for a prolonged period of time to doses of UV-radiation that completely darken ionic AgCl used as control photosensitive substance. That means that fine powders of AgL survive intact during UV-irradiation sufficient for polymerization of the acrylate composite material, which opens the possibility for their further development for in vivo experiments and preclinical and clinical studies. The origin for such significant resistance toward visible and UV-light is in the structures of complexes. In order to show light insensitivity a compound should: (a) form a coordination polymer organized in a manner of zigzag chains or 2D sheets; (b) contain at least two short covalent bonds between silver(I) and nitrogen atoms of the ligand; (c) contain a bridging oxime/nitroso group. Therefore, based on six established up-to-date crystal structures of light stable Ag-cyanoximates (four in this work and the other two are Ag(BCO)<sup>61</sup> and Ag(CCO),<sup>41</sup> the light-insensitive motif can be deduced in oxime-based silver(I) complexes to one in Figure 6.

After many hours of intense short-wavelength UV radiation powdery samples of Ag-cyanoximates eventually become darkened. It should be noted that for the purpose of intended application of synthesized



**Figure 5.** Resistance of synthesized solid silver(I) cyanoximates Ag(CCO), Ag(ACO), Ag(DCO), and Ag(PiCO) toward high-energy UV-radiation at 254 nm in comparison to conventional light-sensitive silver chloride.



**Figure 6.** “Light insensitive motif” in Ag(I) cyanoximates. Solid red lines – absolutely necessary to have bridging nitroso-group: 6 out of 6 cases; dashed red line – binding of silver atom via the cyano-group, and formation of a second bridge via the oxygen atom of the nitroso group: both present in 4 out of 6 cases.

compounds – as antimicrobial light-insensitive additives to adhesives for indwelling medical devices – only minutes of light stability are required. The source of a dark color in final products of degradation is metallic silver that is in the colloidal state. This conclusion is based on comparative analysis of the XRD patterns of fine metallic silver powder (obtained from substitution reaction between Al wire and AgNO<sub>3</sub> aqueous solution at 293 K, pH = 6.0) and those for the initial Ag-cyanoximates, and same complexes darkened after UV-light exposure. Thus,

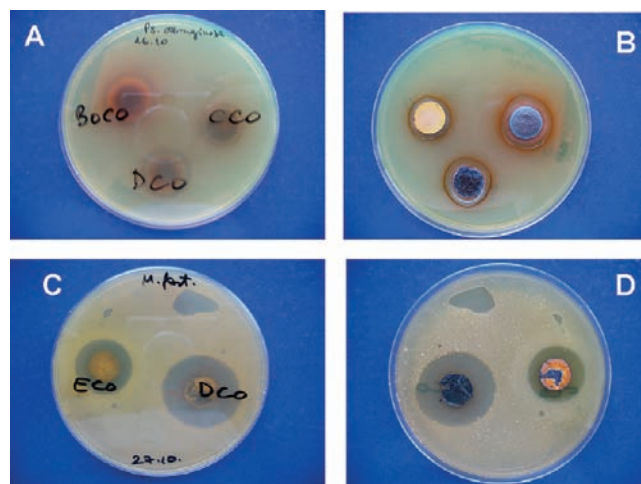
**Table 3.** MIC of Silver(I) Cyanoximates in Solutions<sup>a</sup>

AgL	MIC						
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>S. mutans</i>	<i>S. aureus</i>	<i>M. fortuitum</i>	<i>C. albicans</i>
Ag(ACO)	0.031	1	0.125	0.5	0.063	0.031	0.031
Ag(BCO)	0.031	0.063	0.125	0.5	0.063	<b>0.016</b>	0.031
Ag(BIHCO)	0.25	1	0.5	1	0.5	0.125	0.25
Ag(BIMCO)	0.031	0.031	0.125	1	0.25	0.063	0.031
Ag(BOCO)	0.063	0.031	0.125	0.063	0.063	0.016	0.063
Ag(BTCO)	0.031	0.031	0.25	<i>d</i>	0.125	0.063	0.063
Ag(CCO)	0.031	0.031	0.125	0.063	0.063	0.031	0.063
Ag(DCO)	<b>0.016</b>	0.031	0.063	0.031	<b>0.031</b>	<b>0.016</b>	0.063
Ag(ECO)	0.031	0.031	0.125	0.031	<b>0.031</b>	<b>0.016</b>	0.063
Ag(PICO)	<b>0.016</b>	<b>0.016</b>	0.063	0.031	0.063	<b>0.016</b>	0.063
erythromycin <sup>b</sup>	ne <sup>c</sup>	ne	1	<b>0.004</b>		1	0.25

<sup>a</sup> The lowest MICs are shown in bold. <sup>b</sup> Erythromycin (antibiotic acting against Gram-positive bacteria). <sup>c</sup> ne – not examined. <sup>d</sup> No inhibition effect was detected.

no lines corresponding to metal Ag were observed in the  $\Theta$ – $2\Theta$  XRD scans (Figures S13 and S14, Supporting Information). This is attributed to two factors: (1) to the small size of silver particles estimated to be less than  $\sim 100$  Å, and (2) small physical quantity of produced metallic silver. A typical sample mass of initial AgL spread over the cardboard used in the UV-irradiation studies did not exceed 40–50 mg (Figure S8, Supporting Information). However, characteristic peaks of “plasmon” bands<sup>69</sup> of colloidal silver at 380–450 nm were detected in UV–visible spectra of exposed Ag-cyanoximates in DMSO or Py (Figures S15–S20, Supporting Information) in addition to the Tyndall effect (Figure S21, Supporting Information). Both these features showed colloidal state of metal present in UV-light exposed samples. Studies on the origin of darkening of exposed to UV-light exposed samples and physical properties turned out to be very interesting itself and have developed into a separate investigation, which is out of scope of this paper. Together with data of thermal analysis, it will be published separately elsewhere.

**Biological Part: In Vitro Assessment of Antibacterial Activity of Silver(I)-cyanoximates. Studies in Solutions.** All 10 cyanoximates exhibited antimicrobial activity against the tested infection agents. The lowest MIC value was detected for Ag(DCO) and Ag(PICO), which indicated their highest activity (Table 3). These data suggested that hydrophobic methylated cyanoximes (Scheme 2) showed stronger inhibitory effect possibly due to their interactions with cell membranes which may help in complexes' intracellular uptake. However, the solubility of silver(I) cyanoximates in DMSO and DMF is limited, and, therefore, there was a need in conducting experiments with solid samples of synthesized AgL. This was achieved by using dried paper disks presoaked with AgL solution. Seven out of 10 compounds were studied in this manner. Investigated compounds soluble in DMF were remarkably more effective against clinical isolates from nosocomial infections than to strains from the Collection of Microorganisms.



**Figure 7.** Photographs of agar plates showing zones of inhibition by AgL pellets in two cultures on solid media: in *Pseudomonas aeruginosa* (A - bottom view, B - top view) and in *Mycobacterium fortuitum* (C - bottom view, D - top view) growth on solid media.

**Solid State Studies.** Seven cyanoximates (Ag(BCO), Ag(CCO), Ag(DCO), Ag(ECO), Ag(BIMCO), Ag(BOCO), and Ag(PiCO)) were deposited on filter papers and tested for their effect on bacterial growth on solid media. The inhibition zones of microbial growth was examined and presented in Figure S11, Supporting Information. The results demonstrated that studied Ag(I) cyanoximates exhibit antimicrobial activity in the solid state as well, but their efficiency was strain dependent. Furthermore, we tested the antimicrobial effect of the silver(I) cyanoximates deposited as pressed pellets (12 mm in diameter) against five strains of microorganisms grown on agar plates. Figure 7 shows two examples of growth inhibition experiments and demonstrated clear inhibition zones in *Pseudomonas aeruginosa* growth and *Mycobacterium fortuitum* in the presence of Ag(BOCO), Ag(CCO), Ag(DCO), and Ag(ECO) pellets. The quantitative data are summarized in Figure S12 (Supporting Information), and further confirm that all seven tested silver(I) cyanoximates inhibit bacterial growth and can be used as agents to prevent infections and biofilm formations.

## Conclusions

Ten silver(I) cyanoximates were synthesized and characterized using elemental analyses, spectroscopic methods, and

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X-ray analysis. Five complexes were obtained and characterized for the first time, while crystal structures were determined for four compounds. All synthesized Ag(I) complexes have demonstrated thermal stability upon heating up to 150 °C and a remarkable light insensitivity. That includes years of exposure to daylight or hours of direct exposure to the short wavelength (254 nm) UV-radiation. The antimicrobial activity of all obtained silver(I) cyanoximates was tested both in solutions and in the solid state against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* sp., *Pseudomonas aeruginosa*, *Enterococcus hirae*, *Streptococcus mutans*, *Staphylococcus aureus*, *Mycobacterium fortuitum*, *Candida albicans*, and in addition on several multidrug-resistant bacterial strains isolated from patients with nosocomial infections *K. pneumoniae* 244, *P. aeruginosa*, *Streptococcus pneumoniae* PCI, AMA, *Enterococcus faecium* VRE (vancomycin-resistant), *S. aureus* MRSA (methicillin-resistant). Examination of fresh clinical isolates from nosocomial infections revealed the data valuable for application purposes of the investigated compounds. Results have indicated pronounced antimicrobial activity of studied complexes. A combination of four properties including (1) thermal stability, (2) light insensitivity, (3) poor water solubility, and (4) antimicrobial activity is important for their intended potential applications in indwelling medical devices. Thus, visible light insensitive silver(I) complexes with antimicrobial activity will offer benefits as an adjunct or alternative material compared to current materials used for implants. Inertness of these metal compounds toward intense UV-light allows their application as additives to UV-radiation curable polymeric glues in joint replacement therapy and dental implant insertions. Because bacterial adhesion and biofilm formation are important predisposing factors in the development of clinical implant infection, it is crucial that silver(I) cyanoximates showed an effect on their development. A significant thermal resistance of these complexes is essential for equipment sterilization, while poor water solubility will prevent compounds from leaching out from solidified polymeric composites, or from the surface of indwelling devices. Silver(I) complexes introduced in polymeric acrylate glue composites will prevent infection from occurring.

#### Future Plans

We will investigate mixtures of silver(I) cyanoximates with conventional acrylate-based light-curable glues used in

medical practice during introduction of indwelling devices and conduct light-induced bulk polymerization. Samples for these studies will be in the form of disks or small cylinders in order to model conditions and shapes for indwelling medical devices. Mixtures of silver(I) cyanoximates could also be also tested to ensure broader microbicidal activity. Since preliminary experiments demonstrated that Ag(I) cyanoximates inhibit microbial growth in liquid and solid media, these complexes may prevent or inhibit bacterial adhesion and biofilm formation. Thus, the effect of Ag(I) complexes on adhesion of *P. aeruginosa*, *S. aureus*, and *S. mutans* will be tested, and then the ability of bacteria to develop biofilm on the coated surfaces will be investigated as well.

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**Supporting Information Available:** Actual photographs of crystals of AgL (S1); details of crystal structure determination for Ag(ACO) (S2); table of bonds and angles around silver(I) atom in the structure of Ag(ACO) (S3–4); table of bonds and angles around silver(I) atom in the structure of Ag(DCO) (S5); table of bonds and angles around silver(I) atom in the structure of Ag(PiCO) (S6); table of bonds and angles around metal center in the structure of Ag(ECO) (S7); experimental setup for the photodegradation studies (S8); schematic representation of application of light-stable antimicrobial Ag(I) complexes as glue additive during prosthetic joints introduction (S9); visible spectra of suspensions of two compounds (S10); tables of paper disks and solid pellets studies antimicrobial studies of synthesized AgL (S11, S12); XRD patterns for several studied complexes and metallic silver as a control (S13, S14); UV–visible spectra of some AgL samples in DMSO and Py prior and after their exposure to UV-light (S15, S18); full line shape analysis for the Ag(ACO) (S16, S17) and Ag(ECO) (S19, S20) systems selected as examples; the Tyndall effect in those solutions (S21); calculated from single crystal data and experimental powder XRD patterns from some bulk AgL (S22, S23). This material is available free of charge via the Internet at <http://pubs.acs.org>.